Isolation of Rutin from Tomatin Concentrates

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Introduction

In attempts to purify tomatin, an antibiotic agent that occurs in the tomato plant (1, 2, 3), by chromatographic methods, crystalline rutin has been isolated from certain fractions possessing high tomatin activity. It has been shown, however, that rutin does not exhibit antibiotic properties when tested against the fungus, *Fusarium oxysporum* f. *lycopersici* (R-5-6), which is used as the assay organism for tomatin. On the other hand, rutin is known to antagonize the bacteriostatic activity of dicoumarol toward *Staphylococcus aureus* (4).

Rutin (3,5,7,3′,4′-pentahydroxyflavone-3-rutinoside) is recognized as one of the most widely distributed glycosides, since it has been isolated from 39 species of plants. Nevertheless, the only report of the isolation of this glycoside from the tomato plant is that of Blount (5) who, by the rather ingenious method of carefully wiping the stems of tomato plants with a linen cloth, collected the yellow exudate formed on the stems, and from it obtained crystalline rutin. At the Eastern Regional Research Laboratory attempts to isolate rutin from fresh green tomato leaves, stems and green fruit of commercial tomato varieties by the customary methods have been unsuccessful.

In the work reported here rutin has been isolated from the dried leaves of the Red Currant tomato plant (*Lycopersicon pimpinellifolium*) This tomato variety, which produces currant-sized fruit, is almost immune to the disease known as Fusarium wilt and for this reason it

has assumed considerable importance in the development of wilt-resistant tomato varieties.

Experimental

Material

Red Currant tomato vines, grown to maturity in the field at the Plant Industry Station, Beltsville, Md., were harvested and transported by truck to the Eastern Regional Research Laboratory for drying. Approximately 24 hours elapsed between harvesting and drying, which was accomplished by feeding the chopped vines into a forced draft continuous dryer operating at 105°C. and regulated to discharge the dried material one-half hour after entry. The dry leaves and stems were separated in an air separator and the leaves were then ground to pass a 20-mesh sieve. The ground leaves were used for tomatin and rutin investigations.

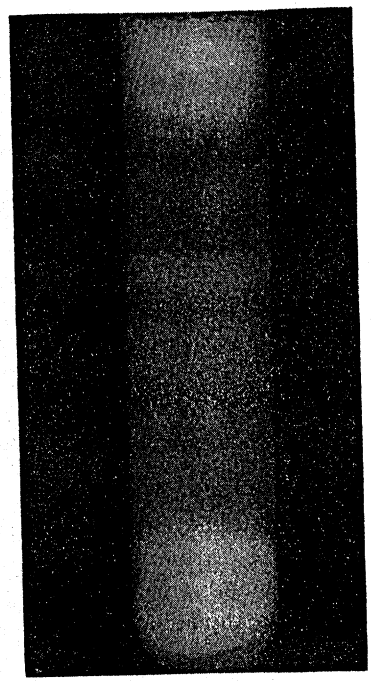

Fig. 1. Chromatographic fractionation of a tomatin concentrate. (Potato starch column; solvent, Butanol-water.) Tomatin and rutin are present in the leading zone.

Procedure

Dried Red Currant tomato leaves (600 g.) were extracted with hot 95% methanol until most of the green color was removed. The methanol extract was concentrated under reduced pressure to a thick syrup, diluted with 1 liter of water, mixed and autoclaved at 15 lbs. steam pressure for one-half hour. After autoclaving, the solution was allowed to cool to permit most of the chlorophyll, gums and lipids to settle to the bottom of the flask, and the supernatant solution was decanted, centrifuged and concentrated to 50 ml. (The tomatin activity, as previously defined (1), of this concentrate was approximately 200 units/ml.) The 50 ml. of tomatin concentrate was shaken vigorously with 50 ml. of normal butanol, resulting in an almost equal distribution of tomatin activity between the two phases. The butanol layer was removed and placed on a potato starch column prepared as follows: Potato starch (800 g.) was ground with 200 ml. of normal butanol. Butanol saturated with water was added to make a slurry. The potato starch slurry was poured into a glass column (5 cm. diam.) and allowed to settle by gravity. The completed starch column was approximately 60 cm. in length and was washed with butanol saturated with water before the addition of the butanol-tomatin extract. Development of the chromatogram with butanol saturated with water resulted in the separation of some of the coloring matter as a zone near the top of the column as is illustrated in the small column pictured in Fig. 1. Tomatin and rutin were found to occur primarily in the leading zone of the column.

The zones were washed from the column with butanol saturated with water at a flow rate of approximately 12 ml./hr. Samples of the eluate, collected at intervals after color began to appear, were concentrated to dryness and then taken up in hot water. These aqueous solutions were assayed for tomatin activity and the remaining

TABLE I

Isolation of Crystalline Rutin from Chromatographic Fractions of a Tomatin Concentrate (Potato Starch Column)

Fraction No.	Butanol-water eluate	Tomatin activity	Rutin crystallized
	ml.	*units/ml. eluate*	
1	180	6.1	yes
2	10	5.3	yes
3	10	4.9	yes
4	15	4.8	yes
5	10	4.3	yes
6	15	4.1	yes
7	12	3.8	no
8	15	3.4	no
9	160	3.1	no
10	70	1.4	no
11	150	1.5	no
12	50	1.1	no
13	200	1.1	no
14	120	0.8	no

solutions were allowed to stand until rutin crystallized spontaneously in those fractions where it was present in sufficient amount (Table I).

On standing in the refrigerator overnight, rutin crystallized from the water solutions of chromatographic fractions 1 through 6 but only a few crystals appeared in fraction 6. The water solutions of chromatographic fractions 7 through 14 were colored but rutin did not crystallize in any case, even after concentration to a very small volume.

Chromatographic fractions 1 through 6 exhibited identical tomatin activity before and after the crystallization and removal of rutin, indicating that rutin exerts no effect upon the tomatin assay. This conclusion has also been repeatedly confirmed by adding pure rutin to solutions of known tomatin activity.

Identification of Rutin

Approximately 175 mg. of crude crystalline rutin was obtained from chromatographic fractions 1 through 6, the largest amount being obtained from fraction 1. The crude rutin, recrystallized once from hot water, melted at 180–190°C. The ultraviolet spectrophotometric method of Porter *et al.* (6), which involves the measurement of spectral densities of a solution of the anhydrous sample in 95% ethanol, was used for final identification. The spectrum was characterized by absorption maxima near 362.7 and 257.7 mμ, with specific extinction coefficients 32.3 and 37.5 liter $g.^{-1}$ $cm.^{-1}$, respectively, and an extinction ratio of 0.883 for wave lengths 375.2 and 362.7 mμ. Highly purified anhydrous rutin has been found by Porter *et al.* (6) to have maxima near 362.7 and 257.7 mμ, with a specific extinction coefficient of 31.9 at 362.7 mμ and an extinction ratio of 0.875 at 375.2 and 362.7 mμ. The results of absorption measurements in the visible range indicated the complete absence of chlorophyll and the presence of some material, soluble in absolute ethanol, which suppresses the curve in the region of 450–660 mμ.

Discussion

To determine the amount of rutin present in Red Currant tomato leaves, 500 g. of the same sample used in the chromatographic work reported above was subjected to the procedure developed at the Eastern Regional Research Laboratory for the isolation of rutin. The rutin isolated amounted to 0.037% (moisture-free basis) of the dried Red Currant tomato leaves. This percentage may be considered a minimum value for Red Currant tomato leaves, since it is possible that: (a) enzymatic degradation of rutin may have occurred during the 24-hour period between the harvesting and drying of the plants; (b)

rutin may have been partially destroyed during the drying process; and (c) the tomato plants may not have been harvested at the stage of growth of maximum rutin content. That these factors have an important bearing upon the rutin content of buckwheat has been shown in the detailed studies at the Eastern Regional Research Laboratory (7).

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Summary

A measurable amount of rutin is present in the leaves of the Red Currant tomato plant (*Lycopersicon pimpinellifolium*). Rutin crystallizes readily from chromatographic fractions having high tomatin activity but rutin does not inhibit the organism (*Fusarium oxysporum* f. *lycopersici*) used for tomatin assay nor does its presence in solution with tomatin appear to influence the assay for tomatin.

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